Institute Report No. 279

Dermal Sensitization Potential of Niclosamide in Guinea Pigs

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

September 1988

Toxicology Series: 233

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal Sensitization Potential of Niclosamide in Guinea Pigs (Toxicology Series 233)-- Frost *et al.*

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 16 Sept 88

Edwin S. Beatrice **(date)**
COL, MC
Commanding

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No. 279	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Branch Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-UL-TO-M	7a. NAME OF MONITORING ORGANIZATION US Army Medical Materiel Development Activity
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5009
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63807A; PROJECT NO. 808; TASK NO. EB; WORK UNIT ACCESSION NO. -

11. TITLE (Include Security Classification)
Dermal Sensitization Potential of Niclosamide in Guinea Pigs (U)

12. PERSONAL AUTHOR(S) Denzil F. Frost, Gary M. Zaucha, Robert E. Harris, C. Dahlem Smith, Conrad R. Wheeler, and Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM 2/16/88 TO 4/18/88	1988 September	29

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Dermal sensitization, Niclosamide, Schistosome topical antipenetrant lotion, Guinea pigs, Guinea Pig Maximization Test

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Niclosamide, the active ingredient in a schistosome topical antipenetrant lotion, was tested for its potential to produce dermal sensitization. The guinea pig maximization test was used to evaluate dermal sensitization potential. Grade III (moderate) dermal sensitization to the niclosamide was obtained in this study. These results suggest that there is probable risk that the active ingredient in the schistosome topical antipenetrant lotion will induce contact sensitivity in humans.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Edwin S. Beatrice, COL, MC	22b. TELEPHONE (Include Area Code) 415-561-3600 22c. OFFICE SYMBOL SGRD-ULZ

ABSTRACT

Niclosamide, the active ingredient in a schistosome topical antipenetrant lotion, was tested for its potential to produce dermal sensitization. The guinea pig maximization test was used to evaluate dermal sensitization potential. Grade III (moderate) dermal sensitization to the niclosamide was obtained in this study. These results suggest that there is probable risk that the active ingredient in the schistosome topical antipenetrant lotion will induce contact sensitivity in humans.

KEY WORDS: Dermal Sensitization, Niclosamide, Schistosome Topical Antipenetrant Lotion, Guinea Pig Maximization Test, Guinea Pigs

PREFACE

TYPE OF REPORT: Dermal Sensitization GLP Report

TESTING FACILITY:
U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:
U.S. Army Medical Research and Development Command
U.S. Army Medical Materiel Development Activity
Fort Detrick, Frederick, MD 21701-5009

TASK AREA: 808EB Schistosome Topical Prophylaxis System

GLP STUDY NO.: 88005

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ, MSC

PRINCIPAL INVESTIGATOR: Denzil F. Frost, MS, DVM, CPT, VC

CO-PRINCIPAL INVESTIGATORS: Gary M. Zaucha, DVM, CPT, VC
Robert E. Harris, DVM, CPT, VC

PATHOLOGIST: C. Dahlem Smith, DVM, MAJ, VC, Diplomate, American Society of Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Niclosamide

INCLUSIVE STUDY DATES: 16 February - 18 April 1988

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of niclosamide in the guinea pig maximization test.

ACKNOWLEDGMENTS

Yvonne C. LeTellier, BS, provided statistical and research assistance; SGT Tammie Heineman, SP4 Barbara Green, SP4 Vilmar Villa, and Rick Katona provided animal care and facility management; Synitha Fuller provided office management during performance of this study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 88005 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

DON W. KORTE, JR., PhD /DATE
MAJ, MSC
Study Director

DENZIL F. FROST, MS, DVM /DATE
CPT, VC
Principal Investigator

CONRAD R. WHEELER, PhD /DATE
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Analytical Chemist

GARY M. ZAUCHA, DVM /DATE
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C. Dahlem Smith, DVM
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Co-Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 22 July 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 88005

1. This is to certify that in relation to LAIR GLP Study 88005, the following inspections were made:

29 January 1988	-	Protocol Review
24 February 1988	-	Animal Receipt
01 March 1988	-	Randomization
21 March 1988	-	Compound Preparation
21 March 1988	-	Weighing
22 March 1988	-	Intradermal Dosing
29 March 1988	-	Topical Dosing
12 April 1988	-	Challenge Dosing
14 April 1988	-	Test Chemical Log
14 April 1988	-	Scoring
18 April 1988	-	Necropsy

2. The institute report entitled "Dermal Sensitization Potential of Niclosamide in Guinea Pigs," Toxicology Series 233, was audited on 29 June 1988.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Dermal Sensitization Potential of Niclosamide in Guinea Pigs-Frost *et al*

INTRODUCTION

The U.S. Army Medical Research and Development Command in collaboration with Miles Pharmaceuticals is developing a Topical Antipenetrant (TAP) lotion to protect U.S. military personnel from schistosomiasis. The Division of Toxicology, LAIR, evaluated the dermal sensitization potential of the TAP lotion in the guinea pig maximization test (GPMT) and concluded that the TAP lotion was a mild sensitizer (1). However, this study was not conclusive for two reasons: First, the vehicle, TAP lotion without active ingredient, was also a mild sensitizer (1), and second, the GPMT used to evaluate the TAP lotion is not as robust an assay for formulations as for the neat compound (2). Therefore, to define the dermal sensitization potential of the TAP lotion more completely, neat niclosamide, the active component of the TAP lotion, was evaluated in a second assay.

Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of niclosamide in the guinea pig maximization test.

MATERIALS

Test Substance

Name: Niclosamide

Chemical Abstracts Registry Number: 50-65-7

LAIR Code Number: TP94

Source: Mr. Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC 20307-5100
Code #: WR 46,234AH

Vehicle: Propylene glycol (J.T. Baker, Phillipsburg, NJ 08865) was used as the test substance vehicle for intradermal induction doses. The expiration date for this lot (501601) was 1 January 1997. Petrolatum white USP (Moyco Industries Inc., Philadelphia, PA 19132/lot # 6503) was used as the vehicle for the topical induction and challenge doses.

Other test substance information is presented in Appendix A.

Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)/Lot# 11F0543

Chemical Abstract Service Registry No.: 97-00-7

Source: Sigma Chemical Company
St. Louis, MO 63178

Vehicle: The vehicle for DNCB was a propylene glycol (3%) and sterile water (97%) mixture. Propylene glycol was from the same lot as for the niclosamide intradermal induction doses. Sterile water (Lot No. 01-075-FW, expiration date 1 Feb 89) was obtained from Abbott Laboratories (North Chicago, IL).

Preparation of Stock Positive Control Solution: The DNCB solution was prepared by first adding 30 mg DNCB to 1 ml propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of sterile water was added to give a concentration of 0.1% (w/v). The solution was heated to 50-60°C and vortexed to keep the DNCB in solution.

Other positive control substance information is presented in Appendix A.

Negative Control

Name: Propylene Glycol

Manufacturer: J.T. Baker Chemical Company
Phillipsburg, NJ 08865

Lot Number/expiration date: 501601/1 Jan 97

Other Study Compounds

Freund's complete adjuvant (FCA) was obtained from Sigma Chemical Company, St. Louis, MO 63178, Lot No. 67F-8834. Propylene glycol, from the same lot as for niclosamide, and

sterile water, from the same lot as for DNCB stock solution, were used to prepare the dilutions of FCA.

Sodium lauryl sulfate (SLS) was obtained from the Sigma Chemical Company, St. Louis, MO 63178, Lot No. 116F-0012. Petrolatum, White, USP (Moyco Industries, Philadelphia, PA 19132, Lot No. 6503) was used as the vehicle for the SLS. The SLS was prepared as a 10% concentration in the petrolatum.

Animal Data

Sixty-seven male guinea pigs, Hartley strain (Simonsen Laboratories, Inc., Gilroy, CA), were used for this study. Two animals were selected randomly for quality control necropsy evaluation on receipt. Five of the animals were used for a pilot study to determine the highest tolerated concentration for the intradermal induction dose, a slightly irritating concentration for the topical induction dose, and a non-irritating concentration for the topical challenge dose. Animal weights on receipt (16 Feb 88) ranged from 174 to 300 g. Additional animal data are presented in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow, Diet #5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water, purified by reverse osmosis, was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 21.1°C to 24.4°C and relative humidity in a range of 28 to 67%, with occasional spikes as high as 93% during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-112, "Guinea Pig Maximization Test" (3), which was based on the method of Magnusson and Kligman (4).

Group Assignment/Acclimation

The guinea pigs were quarantined for 15 days. In addition, 13 days were required for the completion of pilot studies. During the quarantine period, the guinea pigs were

checked daily for signs of illness and weighed once a week. Twenty animals were assigned to each of three groups by a stratified randomization technique based on their body weights in accordance with LAIR SOP OP-STX-78, "Stratified Randomization" (5).

Dosage Levels

The maximum tolerated concentrations for intradermal injections of the niclosamide, DNCB, and FCA were determined to be a 5% concentration of each stock solution.

Both the niclosamide and stock solution of DNCB were shown to be non-irritating when administered topically. Therefore, for the topical induction and the challenge doses, niclosamide was administered 25% by weight in petrolatum and DNCB was administered as the undiluted 0.1% stock solution.

Compound Preparation

Intradermal Induction:

A 5% solution of the niclosamide was prepared by combining 250 mg of the neat compound with 5.0 ml of propylene glycol. Freund's complete adjuvant stock solution was prepared by mixing 7.5 ml of FCA with 7.5 ml sterile water. The DNCB intradermal injection solution was prepared by diluting 0.25 ml of the stock 0.1% DNCB solution with 4.75 ml of propylene glycol. The FCA (5%) and niclosamide (5%) combination was prepared by adding 250 mg niclosamide and 0.25 ml FCA stock to 4.75 ml of propylene glycol. The FCA (5%) and DNCB (5%) combination was prepared by adding 0.25 ml of DNCB 0.1% solution and 0.25 ml FCA stock to 4.5 ml of propylene glycol. Propylene glycol was used for the negative control intradermal injection solution. The FCA (5%) and negative control combination was prepared by adding 0.25 ml of FCA stock to 4.75 ml of propylene glycol.

Topical Induction and Challenge:

The sodium lauryl sulfate (SLS) was prepared by weighing 2.7 g SLS and 24.3 g petrolatum. The two compounds were hand mixed with a spatula.

The niclosamide topical patch was prepared by combining 3.0 g niclosamide with 9.0 g petrolatum and applying 0.5 ml of this mixture to a 2 x 4 cm piece of Whatman #2 filter paper (Whatman, Ltd, New England). The DNCB topical patch

prepared by applying 0.5 ml of DNCB 0.1% solution to a 2 x 4 cm piece of Whatman #2 filter paper. DNCB solutions were prepared fresh for each application day. The negative control topical patch was prepared by applying 0.5 ml of the petrolatum to a 2 x 4 cm piece of Whatman #2 filter paper. The challenge vehicle control topical patch for the DNCB group was prepared by mixing 1 ml propylene glycol with 29 ml sterile water (Abbott Laboratories, North Chicago, IL 60064) and applying 0.5 ml of this solution to a 2 x 4 cm piece of Whatman #2 filter paper.

Test Procedures

Intradermal Injections:

Twenty-four hours before intradermal dosing, an area of 4 x 6 cm over the shoulder of each animal was clipped (Oster®, Model A5, size 40 blade, Sunbeam Corporation, Milwaukee, WI) and then shaved with a disposable razor (Davol, Inc., Cranston, RI) and tap water.

On each shoulder a row of 3 injections (1 cc syringe, Lot 1 D017, Becton Dickson; 26-gauge, 0.5-in. needle, Becton Dickson), six injections in all, were made as follows: 1) 0.1 ml of the adjuvant alone, 2) 0.1 ml of test substance/DNCB/vehicle without adjuvant, and 3) 0.1 ml of the test substance/DNCB/vehicle emulsified in FCA (final concentration 5%) for the test compound/positive control/negative control groups. All injections were made deep into the dermis to minimize sloughing.

Topical Induction Application:

One week after the injections and 24 hours before the topical application, the 4 x 6 cm area was clipped again and shaved closely with a disposable razor and tap water. Since topical administration of the test agent was non-irritating, the area was pretreated with 10% sodium lauryl sulfate in petrolatum immediately after the clipping and shaving. The SLS was massaged into the skin with a glass rod, and the skin was not covered. The residual SLS was removed the next day with gauze just prior to topical induction.

A 2 x 4 cm patch of Whatman # 2 filter paper was saturated with approximately 0.5 ml of the appropriate test or control substance. The patch was covered by overlapping with an occlusive tape (Blenderm®, Medical Products Division/3M, St. Paul, MN 55144, Lot 273) approximately twice the size of the patch. The occlusive tape was firmly secured by elastic

adhesive bandage (Conform®, the Kendall Company, Hospital Products, Boston, MA 02101-5229, Lot # 521018). This dressing was left in place for 48 hours. Care was taken to ensure that the wraps remained in place throughout the application period.

Challenge Application:

Test compound and negative control animals were challenged topically with niclosamide two weeks after the topical induction. Positive control animals were challenged with DNCB 2 weeks after the topical induction. Hair was removed from a 5 x 5 cm area on each flank by clipping and shaving as for the induction phase. The test substance (approximately 0.5 ml) was applied on a 2 x 2 cm piece of Whatman # 2 filter paper and sealed to the left flank as for the induction phase. A second filter paper was used to apply an equal volume of the vehicle to the contralateral flank in the same manner as the test substance. The patches and occlusive tape were firmly secured by elastic adhesive bandage (Conform®, The Kendall Company, Hospital Products, Boston, MA 02101, Lot # 521018). The patches were left in place for 24 hours.

Reading the Challenge Reactions:

The challenge reaction was evaluated 24 hours after removal of the patch. Any irritation produced by the plastic tape would be expected to have subsided by then so that any reaction observed could be attributed to an allergic response. The sites were examined again at 48 hours after removal of the patch, primarily to detect weak or slowly developing reactions.

Reactions were scored on a 4-point scale: no reaction, 0; scattered mild redness (erythema), 1; moderate and diffuse redness, 2; and intense redness and swelling, 3. A response was positive if it exceeded the response on the contralateral side and there was no more than a slight redness attributable to the test substance in the negative control group.

Necropsy

All guinea pigs were submitted for a complete gross necropsy at the conclusion of the 27-day observation period.

Duration of Study

A historical listing of study events appears in Appendix C.

Deviations from Protocol

Shaving was done with disposable razors and water rather than an electric razor in order to obtain a more satisfactory clean-shaven area. Animals 88E0021, 110, and 112 died prior to completion of the study. Consequently, the final group numbers for the test compound, positive control, and negative control groups were 20, 19, and 18, respectively. It is believed that these deviations from the protocol did not adversely affect the study results.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Experimental Findings

Table 1 summarizes the incidence of reactions 24 and 48 hours after each challenge dose. The niclosamide produced a positive response, peaking at 48 hours with a 45% incidence.

Table 2 summarizes the severity of skin reactions at 24 and 48 hours. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. This produced a peak severity index of 0.50 at 48 hours for niclosamide.

Table 1

Incidences of Challenge Skin Reactions

Test Group	Hrs After Challenge 24	48
Niclosamide	.35	.45
DNCB Positive Control	.84	.68
Negative Control	.06	.06

Table 2

Average Severity of Challenge Skin Reactions

Test Group	Hrs After Challenge 24	48
Niclosamide	.35	.50
DNCB Positive Control	1.37	1.16
Negative Control	.11	.11

Dinitrochlorobenzene (DNCB) produced a positive response at both time points, peaking at 24 hours. Eighty-four percent of the DNCB-treated animals exhibited a positive response 24 hours following the challenge doses. These reactions diminished, yielding positive effects in 68% of the animals at 48 hours after dosing. The severity scores for the responses to the challenge doses of DNCB were 1.37 at 24 hours and 1.16 at 48 hours. At 24 hours, 6% of the negative control animals (challenge dose of niclosamide) exhibited a reaction. Individual severity scores are tabulated in Appendix D.

Pathology

Three animals were removed from the study because of spontaneous death. Gross and microscopic lesions observed in animals dying were consistent with enterocolitis. *Salmonella* sp. group B was cultured from each of these animals. No gross lesions attributable to the test compound were observed in any of the animals that remained in the study. Pathologic changes consistent with subclinical salmonellosis were observed in the test compound, positive control, and negative control groups at incidence rates of 7/20, 10/19, and 5/18, respectively.

DISCUSSION

Guinea Pig Maximization Test

The guinea pig maximization test is more sensitive than the Landsteiner-Draize or other experimental methods of identifying contact allergens (6). The number of sensitized animals in the test group is an indication of the potency of the contact allergen. Positive patch test reactions may vary considerably in strength. However, it is the total number of positive responses (frequency) and not the intensity which determines the study outcome. The potency of an allergen is based on the percentage of animals sensitized (frequency) according to the following scale (4):

0-8% Weak (Grade I)
9-28% Mild (Grade II)
29-64% Moderate (Grade III)
65-80% Strong (Grade IV)
81-100% Extreme (Grade V)

If more than 10% of the animals are sensitized (Grade II, Mild), there is an obvious risk that sensitization will occur in humans.

Niclosamide

In the present study, niclosamide was evaluated for dermal sensitization potential according to the guinea pig maximization test criteria (4). The study results indicate that the niclosamide sensitized 45% of the animals; this places the compound in the moderate sensitizer category.

Niclosamide is the active ingredient in a schistosome topical antipenetrant lotion. A previous study (1) examined

sensitization potential of the lotion verses a placebo which did not contain niclosamide. The end result of that study indicated that both the lotion and the placebo were mild sensitizers. Wahlberg and Fregent have emphasized that one should assess the sensitizing potential of a formulated product such as the schistosome TAP lotion by conducting the guinea pig maximization test on its individual constituents (2). Their rationale was that the formulation may prevent one from obtaining a sufficient concentration of the active ingredient to induce a sensitizing response in the test system. This would result in a false negative response or an understatement of the active ingredient's sensitizing potential.

The presence of subclinical salmonellosis in the study animals did not appear to compromise the study results. Ten study animals assigned to the positive control group showed evidence of *Salmonella* at necropsy. Nine of these animals also showed a positive response to DNCB. This indicates that the presence of *Salmonella* did not reduce the immune response potential of these respective study animals. In addition, five study animals of the negative control group showed evidence of salmonellosis. None of these animals responded to the challenge dose of the test compound, which indicates that the presence of *Salmonella* did not induce false positive responses. One of the negative control animals (88E0003) did reflect a positive response to the test compound, but did not show evidence of *Salmonella*.

The results of this study indicate that niclosamide, the active component of the schistosome topical antipenetrant lotion, is a moderate sensitizer in guinea pigs. This is a more potent response than obtained with the TAP lotion and may be attributable to the fact that there was a lower concentration of niclosamide in the lotion than was evaluated in the present study.

CONCLUSION

Niclosamide is a moderate sensitizer under the conditions of this study.

REFERENCES

1. Frost DF, Magnuson DK, Korte DW. Dermal sensitization potential of a schistosome topical antipenetrant lotion in guinea pigs. Institute Report No. 261. Toxicology Series 202. Presidio of San Francisco, CA: Letterman Army Institute of Research, 1988.

2. Wahlberg JE, Fregent S. Guinea pig maximization test. In: Maibach HI and Lowe NJ, eds. Models in dermatology, Vol 2. Basel: Karger, 1955; 225-233.

3. Guinea pig maximization test. LAIR Standard Operating Procedure OP-STX-112, Letterman Army Institute of Research, Presidio of San Francisco, CA, 10 September 1987.

4. Magnusson B, Kligman AM. The identification of contact allergens by animal assay. The guinea pig maximization test. J Invest Dermatol 1969; 52: 268-276.

5. Stratified Randomization. LAIR Standard Operating Procedure OP-STX-78, Letterman Army Institute of Research, Presidio of San Francisco, CA, 15 December 1985.

6. Klecak G. Identification of contact allergens: predictive tests in animals. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology and pharmacology. Washington, DC: Hemisphere Publishing Corporation, 1977: 305-339.

Appendix A: CHEMICAL DATA

Chemical name: Niclosamide

Alternative chemical names:

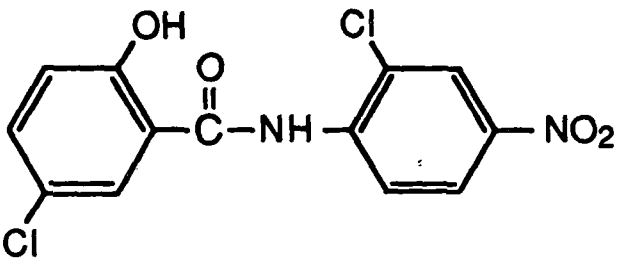
5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide; 2,5-Dichloro-4-nitrosalicylanilide; 5-chloro-N-(2-chloro-4-nitrophenyl) salicylamide; 5-chlorosalicyloyl-(*o*-chloro-*p*-nitranilide); N-(2-chloro-4-nitrophenyl)-5-chlorosalicylamide.

Lot number: 53F-0096

Chemical Abstracts Service Registry No.: 50-65-7

LAIR code number: TP94

Chemical structure:



Molecular formula: $C_{13}H_8Cl_2N_2O_4$

Molecular weight: 327.1

Analytical data:

IR(KBr):3245,3101,1649,1612,1567,1518,1348,1328,1286, 1219,1192,1120,898,829,and 744 cm^{-1}.[1] The IR spectrum was identical to that provided by the sponsors.[2]

HPLC analysis was performed using a Hewlett-Packard 1090 system equipped with a diode array detector. The compound was chromatographed under the following conditions: column, Brownlee RP-18 Spheri-5 (250 x 4.6 mm); mobile phase, methanol/methanol saturated with KH_2PO_4, 50/50 (v/v); flow rate, 1.0 ml/min; wavelength monitored, 330 nm; run-time, 15 min. The compound eluted as one peak with a retention time of 3.9 min.[3]

Appendix A (cont.): CHEMICAL DATA

NMR (300 MHz, d_6-DMSO): 1.14 δ (s, small impurity), 3.22 (s,1H,NH), 7.07 (d, J=8.7 Hz, 2H, CONH-C-C<u>H</u>-CH), 7.52 (d, J=11.3, 2H, CH-C<u>H</u>-Cl), 7.95 (s, 1H, Cl-C-C<u>H</u>-C-CONH), 8.27 (d, J=9.1 Hz, 2H, C<u>H</u>-CH-CONH), 8.30 (s, 1H, Cl-C-C<u>H</u>-NO_2), 8.85 (d, J=9.3 Hz, 2H, HO-C<u>H</u>), 11.50 (s, 1H, OH).[4] The spectrum provided by the sponsors began at 2.50 δ and thus did not show the signal observed at 1.14 δ. All other peaks correspond exactly to the reference spectrum.[2]

Supplied by: Mr. William Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, D.C.

---

[1] Wheeler CR. Military Toxicologic Testing. Laboratory Notebook #85-03-009, p. 11. Letterman Army Institute of Research, Presidio of San Francisco, California.

[2] Masamori E, Benitez A, and Lim P. Assay of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (Niclosamide) in the formulated sols. WR-46234AJ, BL44970 (1% w/v Active) and WR-46234AK, BL44989 (placebo), Report # 586, Project Number 8504, 4 September 1987. SRI International, Menlo Park, California.

[3] Wheeler CR. Miltary Toxicologic Testing. Laboratory Notebook #85-03-009, pp. 15, 16. Letterman Army Institute of Research, Presidio of San Francisco, California.

[4] *Ibid.* pp. 13, 14.

Appendix A (cont.): CHEMICAL DATA

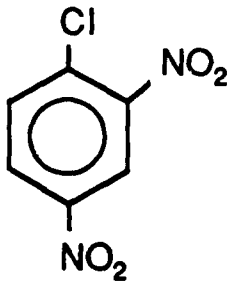
POSITIVE CONTROL

Chemical name: 1-Chloro-2,4-dinitrobenzene

Alternate chemical name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry No.: 97-00-7

Chemical structure:



Molecular formula: $C_6H_3N_2O_4Cl$

Molecular weight: 202.6

Physical state: Yellow crystals

Melting point: 52-54° C[5]

Purity:

The compound was designated as 95% pure by source.

Analytical Data:

Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[6] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (δ).[7] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[8]

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[9] Differences were due to the much finer spectral resolution obtained on the Perkin-Elmer 983 instrument. NMR ($CDCl_3$): δ 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[10]

Appendix A (cont.): CHEMICAL DATA

GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[10]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

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[4] Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

[5] Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[6] *Ibid.* pp 11-12.

[7] *Ibid.* pp 13-16.

[8] Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[9] Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[10] Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Cavia porcellus*

Strain: Hartley

Source: Simonsen Laboratories, Inc.
1180C Day Road
Gilroy, CA 95020

Sex: Male

Date of birth: 26 January 1988, 2 February 1988

Method of randomization: Weight bias, stratified animal allocation (LAIR SOP OP-STX-78).

Animals in each group: 20 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tagging

Pretest conditioning: Quarantine/acclimation 16 Feb - 22 Mar 88

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF EVENTS

Date	Event
16 Feb 88	Animals arrived. Animals were examined, placed in cages, and fed.
17 Feb 88	Animals were ear tagged and weighed. Quality control animals were submitted for necropsy.
17 Feb 88 - 17 Apr 88	Animals were checked daily.
17, 22, 29 Feb 88, 7, 14, 21, 28 Mar 88, 4, 11, 18 Apr 88	Animals were weighed.
1 Mar 88	Animals were transferred from GLP Study 87017.
29 Feb 88	Remaining animals were randomized into groups.
21, 28, Mar 88, 11 Apr 88	All animal were clipped and shaved.
22 Mar 88	All animals were given intradermal induction dose.
29 Mar 88	All animals were given topical induction dose.
31 Mar 88	Topical induction dose dressing and remaining chemical were removed.
12 Apr 88	All animals were given challenge dose.
13 Apr 88	Challenge dose dressing and remaining chemical were removed.
14, 15 Apr 88	All animals were scored for 24-hour and 48-hour skin reactions.
18 Apr 88	All animals were submitted to the Necropsy Suite for sacrifice.

Appendix D: INDIVIDUAL DERMAL SCORES

Niclosamide

Animal Number	Hours Post-Challenge 24	48
(87E00---)		
6	0	0
9	1	1
14	0	0
15	0	0
32	0	1
48	0	0
52	0	0
55	0	0
56	1	1
58	0	0
85	0	0
87	1	1
91	1	2
95	0	0
97	0	0
99	0	0
102	1	1
103	0	1
105	1	1
108	1	1
Incidence of Reaction	.35	.45
Average Severity	.35	.50

Appendix D (cont.): INDIVIDUAL DERMAL SCORES

DNCB Positive Control Group

Animal Number	Hours Post-Challenge 24	48
(87E00---)		
2	2	2
5	0	0
7	0	0
19	1	0
23	2	1
26	1	1
33	0	0
38	1	2
41	2	2
67	2	2
69	2	2
74	2	0
79	2	2
84	1	0
93	2	2
94	2	2
96	2	2
98	1	1
107	1	1
Incidence of Reaction	.84	.68
Average Severity	1.37	1.16

Appendix D (cont.): INDIVIDUAL DERMAL SCORES

Negative Control (Vehicle) Group

	Hours Post-Challenge	
Animal Number	24	48
(87E00---)		
03	2	2
17	0	0
20	0	0
22	0	0
24	0	0
35	0	0
44	0	0
50	0	0
51	0	0
60	0	0
68	0	0
71	0	0
80	0	0
82	0	0
83	0	0
106	0	0
109	0	0
113	0	0
Incidence of Reaction	.06	.06
Average severity	.11	.11

Appendix E: PATHOLOGY REPORT

I. Compound: Niclosamide
Species: Cavia porcellus, Hartley, young adult (200-300 gram body weight range), male

II. Investigators: Denzil Frost, MS, DVM, CPT, VC
Gary Zaucha, DVM, CPT, VC
Robert Harris, DVM, CPT, VC

Pathologist: C. Dahlem Smith, DVM, MAJ, VC

III. Findings: No lesions directly attributable to the application of the compound were observed in these animals. Gross and microscopic lesions observed in those animals dying prior to the scheduled sacrifice (spontaneous deaths) were attributed to septicemia. Salmonella sp., group B, was cultured from three animals.

Gross and microscopic lesions observed in clinically normal animals were either a result of a subclinical infection or were incidental lesions routinely observed in guinea pigs. The subclinical infection manifestations may have been due to a Gram negative septicemia similar to that observed in the spontaneous death animals.

Salmonella, which is carried by many normal guinea pigs, is the most common bacterial infection in guinea pigs. The effect of a subclinical endemic infection on this study is difficult to assess. Direct effects of this infection were evident as necrosis and depletion of many of the lymphoid tissues in the spontaneous death group. The effect of the subclinical infection on the function of the immune system could not be determined.

Lesions of an incidental nature were exemplified by idiopathic hepatic necrosis, subacute salivary gland adenitis, unilateral testicular hypoplasia, the presence of lymphoid tissue in the lung and kidney, and mineralization present in the liver, kidney and stomach. These lesions, except for the Harderian gland adenitis, were observed in all groups, sacrificed at study termination.

In addition, a single skeletal myopathy and a single growth plate abnormality were noted in 2 of the quality control animals. Similar lesions may occur in a variety of animals during their fast growth period or could be the result of a traumatic episode.

C. DAHLEM SMITH, DVM
MAJ, VC
Division of Pathology

9 June 1988

Appendix E (cont.): PATHOLOGY REPORT

Group 1. 20 animals sacrificed on 18 April, 1988

Animal #	Accession #	Gross and Microscopic Observation
88E0006	43113	no gross observations
88E0009	43114	multiple white foci liver surface #1,2,3,4,5,7,8
88E0014	43115	no gross observations
88E0015	43116	no gross observations
88E0032	43117	no gross observations
88E0048	43118	no gross observations
88E0052	43119	.2 x .2 cm white focus liver surface #1,2,3
88E0055	43120	1 x .1 cm white focus liver surface lung lobe consolidation # 1,4,5
88E0056	43121	multiple white foci liver surface #1,2
88E0058	43122	.2 x .2 cm white focus liver surface
88E0085	43123	no gross observations
88E0087	43124	multiple white foci liver surface #1,5,6,7
88E0091	43125	.1 x .1 cm white focus liver surface #1,5,7
88E0095	43126	no gross observations
88E0097	43128	no gross observations
88E0099	43127	no gross observations
88E0102	43129	hypoplasia left testicle
88E0103	43130	no gross observations
88E0105	43131	hypoplasia left testicle
88E0108	43132	multiple white foci liver surface

Appendix E (cont.): PATHOLOGY REPORT

Group 2. 19 animals sacrificed on 18 April, 1988

Animal #	Accession #	Gross and Microscopic Observation
88E0002	43133	multiple white foci liver surface, .1 X .5 cm #1,2,5,8
88E0005	43134	no gross observations
88E0007	43135	.5 x 1 cm white focus liver surface #1,2,3
88E0019	43136	no gross observations
88E0021	43020	see Spontaneous Deaths
88E0023	43137	hypoplasia left testicle
88E0026	43138	multiple white foci liver and spleen surfaces #1,5,6,8
88E0033	43139	no gross observations
88E0038	43140	no gross observations
88E0041	43141	no gross observations
88E0067	43142	multiple white foci liver surface
88E0069	43143	no gross observations
88E0074	43144	multiple white foci liver surface
88E0079	43145	multiple white foci liver surface
88E0084	43146	.3 x .5 cm white focus liver surface
88E0093	43147	no gross observations
88E0094	43148	multiple white foci liver surface #1,5
88E0096	43149	multiple white foci liver surface
88E0098	43150	.2 cm diameter white focus liver surface
88E0107	43151	hydronephrosis left kidney #9

Appendix E (cont.): PATHOLOGY REPORT

Group 3. 18 animals sacrificed on 18 April, 1988

Animal #	Accession #	Gross and Microscopic Observation
88E0003	43152	no gross observations
88E0017	43153	multiple white foci liver surface
88E0020	43154	no gross observations
88E0022	43155	multiple white foci liver surface
88E0024	43156	white lobule liver surface
88E0035	43157	no gross observations
88E0044	43158	no gross observations
88E0050	43159	no gross observations
88E0051	43160	no gross observations
88E0060	43161	4 mm accessory gallbladder
88E0068	43162	no gross observations
88E0071	43163	no gross observations
88E0080	43164	multiple white foci liver surface #1,3,5
88E0082	43165	no gross observations
88E0083	43166	.4 x .8 cm white focus liver surface
88E0106	43167	no gross observations
88E0109	43168	no gross observations
88E0113	43169	no gross observations

Appendix E (cont.): PATHOLOGY REPORT

Animal #	Accession #	Gross and Microscopic Observation
		Spontaneous Deaths
88E0021	43020	gross observations not available #1,4,10,16,25,26,27,28,29
88E0110	43041	emaciation multiple white foci liver surface splenomegaly thickened cecum with white foci #1,5,10,16,17
88E0112	43042	emaciation #1,2,8,16,18,19
		Quality Control
88E0043	42884	.2 x .3 cm white focus liver surface #2,5,7,19,20,21,22
88E0063	42885	.2 x .2 cm white focus kidney surface * #7,23,
88E0073	42886	crusty debris, outer ear canal #19,20

* gross and microscopic re-examination of the specimen indicated the presence of subcapsular fat

GLOSSARY OF MICROSCOPIC LESIONS:

#1 granulomatous hepatitis, multifocal, mild to moderate, liver

#2 coagulative necrosis, multifocal, mild to moderate, liver

#3 portal fibrosis and bile duct hyperplasia, mild to moderate, multifocal, liver

#4 hepatocellular vacuolation, centrolobular, multifocal, minimal to moderate, liver

#5 reticuloendothelial hyperplasia, minimal to mild, spleen and/or lymphoid tissue, with heterophilia

#6 enteritis, acute, mild to moderate, intestine

Appendix E (cont.): PATHOLOGY REPORT

GLOSSARY OF MICROSCOPIC LESIONS (cont.):

#7 lymphoid tissue, paravascular and parabronchiolar, multifocal, minimal to mild, lung

#8 bronchopneumonia, subacute, multifocal, minimal to mild, lung

#9 nephropathy, bilateral, kidney

#10 lymphoid depletion and necrosis, mild to severe, with granuloma formation, lymphoid tissues

#11 epididymitis, suppurative, subacute to chronic, marked, with squamous metaplasia, epididymis

#12 seminal vesiculitis, suppurative, subacute to chronic, mild, seminal vesicle

#13 pyelitis, acute, mild, kidney

#14 myocarditis, subacute, multifocal, mild, heart

#15 hemorrhage, acute, multifocal, mild, cerebellum and medulla, central nervous system

#16 enteritis, ulcerative, necrogranulomatous, acute to subacute, focal to multifocal, moderate, intestine

#17 cholecystitis, subacute, diffuse, mild, gallbladder

#18 glomerulitis, acute, diffuse, mild, kidney

#19 mineralization, multifocal, liver, renal vessels, stomach

#20 adenitis, subacute, multifocal, minimal, Harderian gland

#21 sinus histiocytosis, cortical, lymph node

#22 lymphoid tissue, submucosal, urinary pelvis, minimal, kidney

Appendix E (cont.): PATHOLOGY REPORT

GLOSSARY OF MICROSCOPIC LESIONS (cont.):

#23 myopathy, degenerative, multifocal, minimal, skeletal muscle

#24 chondrodysplasia, focal, minimal, tibial physis, tibia

#25 mineralization and necrosis, multifocal, minimal, heart

#26 hydrocephalus, minimal, cerebral ventricles, brain

#27 dermatitis, exudative, focal, mild, skin

#28 colitis, catarrhal, acute, diffuse, minimal, colon

#29 proteinic tubular casts, multifocal, mild, kidney

* gross and microscopic re-examination of the specimen indicated the presence of subcapsular fat

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